

New species and records of *Endophragmiella* from freshwater and terrestrial habitats in Thailand

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ABSTRACT—Seven *Endophragmiella* taxa were found in Thailand, from freshwater and terrestrial habitats in various locations. Five of them are proposed as new species: *E. acutispora*, *E. filiformis*, *E. obpyriformis*, *E. sporoprolifera*, and *E. tunicata*. *Endophragmiella acutispora* is characterized by fusiform to obclavate, 2–5-septate conidia; *E. filiformis* by obclavate, appendiculate, 3–5-septate conidia; *E. obpyriformis* by its obovoid or obpyriform, bicolored conidia; *E. sporoprolifera* by obclavate and Y-shaped, appendiculate, 5–6-septate conidia; and *E. tunicata* by cylindrical or oblong, tunicate, 2-septate conidia. Two other species, *E. multiramosa* and *E. resinae*, are new records for the Thailand mycota. Detailed descriptions, illustrations, and taxonomic keys comparing the new species with morphologically similar species are provided.

KEY WORDS—Hyphomycetes, morphology, *Sordariomycetes*, *Sordariomycetidae*

Introduction

Endophragmiella B. Sutton was established by Sutton (1973) for *E. pallescens* B. Sutton (type) and *E. canadensis* (Ellis & Everh.) B. Sutton. Subsequently, Hughes (1979) revised *Endophragmiella* based on morphological characteristics of its conidiogenesis, described new species, and updated the species number. Keys to *Endophragmiella* species were provided by Kirk (1985), Holubová-Jechová (1986), Wu & Zhuang (2005), and Xia & al. (2016). More than 90 taxa of *Endophragmiella* are now accepted in the genus (Index Fungorum; <https://www.indexfungorum.org/Names/Names.asp>, accessed 20 April 2023). This genus is mainly characterized by branched or simple conidiophores, monoblastic percurrently extending conidiogenous cells, and variable conidial shape and septation, seceding rhexolytically. *Endophragmiella* species are saprobic and occur on dead plant material, in soils, and in some cases, on *Agaricomycetes*, *Lecanoromycetes*, *Leotiomycetes*, and *Sordariomycetes* as opportunist saprobes that are distributed worldwide in tropical and temperate environments (e.g., Sutton 1973; Hawksworth 1979; Hughes 1979; Kirk 1981a, 1982a, 1983, 1985; Dunn 1982; Holubová-Jechová 1986; Wu & Zhuang 2005; Brackel & Svetlana 2009; Ma & al. 2011, 2012, 2015, 2021a; Zhurbenko & al. 2015; Xia & al. 2016; Jiang & al. 2018).

Species identification in *Endophragmiella* has been problematic due to a lack of molecular data from ex-type cultures and the difficulty of growing specimens on culture media. Identification of *Endophragmiella* species is mainly based on distinct morphological characteristics, especially in conidial form, size, septation, pigmentation, apical filament of the conidia, and conidiophores (Hughes 1979, Holubová-Jechová 1986, Wu & Zhuang 2005).

During the mycological survey of freshwater and terrestrial microfungi on plant debris from Thailand, seven taxa that resemble *Endophragmiella* were isolated, but they could not be successfully cultured. Their morphological characteristics confirmed that these taxa belong to *Endophragmiella*. Two of them were identified as new records from Thailand and the other five are proposed here as new species.

Materials & methods

Collection and morphological data

The collections were made from five freshwater and three terrestrial habitats in Thailand. Naturally decaying and submerged decaying woody specimens were randomly collected and placed in plastic bags, taken to the laboratory, and prepared

according to Chuaseeharonnachai & al. (2013). Woody samples were incubated in a moist chamber at room temperature (~25–27°C) for 7–10 days. Samples were periodically examined for the presence of microfungi under a stereomicroscope (OLYMPUS SZ61; Olympus Corporation, Japan) over 2 months. Several attempts were made to isolate the fungi in pure cultures by transferring single conidia with a fine needle to plates with different media, including potato dextrose agar (PDA; BD Difco™, Becton, Dickinson and Company, USA), corn meal agar (CMA; BD Difco™), malt extract agar (MEA; BD Difco™), potato carrot agar (PCA; HiMedia, HiMedia Laboratories Pvt. Ltd., India) and water agar (WA). Plates were incubated at room temperature for up to 30 days. No viable fungi could be isolated, suggesting that the nutritional requirements were not met by any of the tested media. DNA isolation and amplification from fungal tissues of these studying fungi by using the NucleoSpin® Plant II extraction kit (MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany) was also unsuccessful due to the small amounts of fungal material and the contamination from other microbes. For morphological examination, fungal structures were mounted in a drop of water with lactophenol and examined under higher magnification using a compound microscope (OLYMPUS CX31; Olympus Corporation, Japan). Microfungi were photographed using a Nomarski differential interference contrast microscope (OLYMPUS DP70; Olympus Corporation, Japan). The types and additional specimens were deposited in the BIOTEC Bangkok Herbarium, Pathum Thani, Thailand (BBH) [<https://www.nbt-microbe.org>].

Results

Seven species of *Endophragmiella* were found colonizing dead plant debris. Five of them were identified as new species by morphological characterization: *E. acutispora*, *E. filiformis*, *E. obpyriformis*, *E. sporoprolifera*, and *E. tunicata*. The other two are described and illustrated as new records from Thailand of *E. multiramosa* and *E. resinae*.

Taxonomy

Endophragmiella acutispora Chuaseehar., Somrith. & Boonyuen, **sp. nov.** FIG. 1
IF 558383

Differs from *Endophragmiella acuta* by having larger conidia with more septa; and from *E. selenosporellaria* by its conidia having both basal and apical cells paler than the brown central cells.

TYPE: Thailand, Chiang Mai Province, Mueang Chiang Mai District, a waterfall in Chiang Mai Zoo, 18.8103°N 98.9475°E, on submerged twigs of an unidentified plant, 30 August 2019, N. Boonyuen (**Holotype**, BBH 49151).

ETYMOLOGY: Latin, *acutispora*, referring to the acute conidial apex.

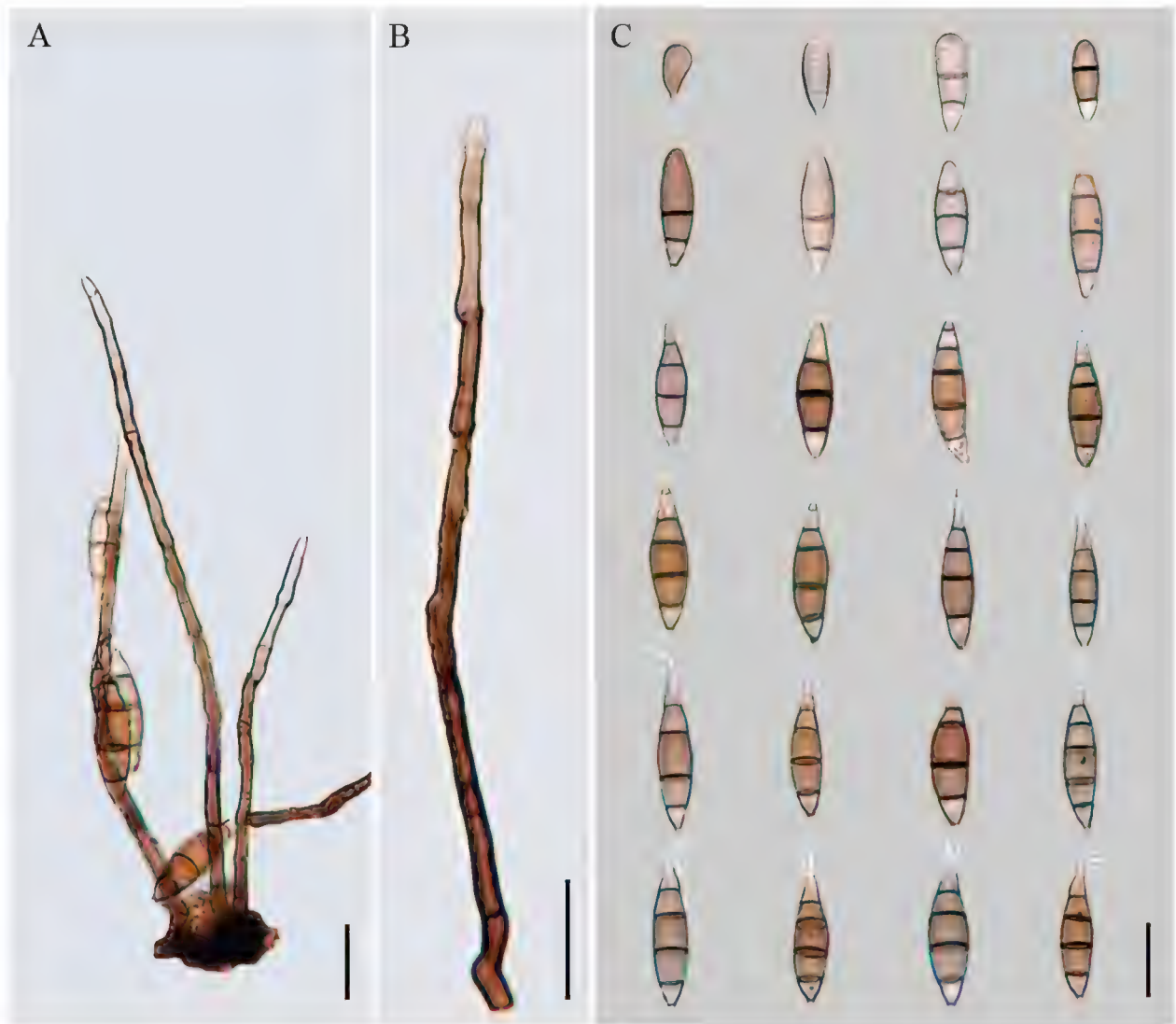


FIG. 1. *Endophragmiella acutispora* (holotype, BBH 49151): A, B. Conidiophores, conidiogenous cells and conidia; C. Conidia at different stages of development, some of which (two bottom rows) bear *Selenosporella*-like synanamorph on the apical cell. Scale bars = 25 μ m.

ASEXUAL MORPH. COLONIES on the natural substratum effuse, hairy, brown. MYCELIUM partially superficial, mostly immersed, smooth-walled, composed of branched, septate, pale brown to brown hyphae, 2.5 μ m diam. CONIDIOPHORES macronematous, mononematous, solitary or in small groups, cylindrical, erect, straight or flexuous, smooth- and thick-walled, simple or occasionally branched, septate, brown, paler near the tip, up to 335 μ m high, 4.5–5 μ m wide at the base, with up to 10 or more percurrent extensions at the upper part. CONIDIOGENOUS CELLS monoblastic, integrated, terminal, percurrent, cylindrical, tapering to a truncate apex, pale brown. CONIDIAL SECESSION rhexolytic. CONIDIA acrogenous, solitary, fusiform or obclavate, rostrate, smooth-walled, (2–)4(–5)-septate, central cells brown, basal and

apical cells paler, (42.5–)47.5–57.5 µm long, 10–12.5 µm wide at the broadest part (avg. = 50.7 × 11.7 µm, n = 50); apex acute and elongated, often bearing synanamorphic conidia; base conical and truncate with a distinct, subhyaline, 2.25–2.5 × 2.5 µm basal frill.

SYNANAMORPH: *Selenosporella*-like. CONIDIA filiform, acerose, aseptate, hyaline, 7.5–12.5 × 1.3 µm, formed on apical cell of the conidia.

SEXUAL MORPH unknown.

ADDITIONAL SPECIMEN EXAMINED—THAILAND, CHIANG MAI PROVINCE, Mueang Chiang Mai District, a waterfall in Chiang Mai Zoo, 18.8103°N 98.9475°E, on submerged twigs of an unidentified plant, 29 August 2019, N. Boonyuen (BBH 49152).

COMMENTS—*Endophragmiella acutispora* resembles *E. acuta* W.P. Wu and *E. selenosporellaria* Y.R. Ma & X.G. Zhang in its fusiform to obclavate conidial shape and its *Selenosporella*-like synanamorph developed from apical cells of the conidia; however, these two species differ by their smaller conidia with fewer septa (*E. acuta*, 18–25 × 8–10 µm, 3 septa, Wu & Zhuang 2005; *E. selenosporellaria*, 14.5–22 × 4.5–6.5 µm, 0–3 septa, Ma & al. 2015) and by apical conidial cells that are paler than the other cells. The conidial shape of *E. acutispora* is also morphologically similar to *E. liquidambaris* Jian Ma & X.G. Zhang, which differs by having an apical mucilaginous appendage and the absence of a *Selenosporella*-like synanamorph and by its smaller conidia with fewer septa (20–26.5 × 9–11.5 µm, 3 septa; Ma & al. 2012).

Key to *Endophragmiella acutispora* and similar species

- 1. Conidia with *Selenosporella*-like synanamorph 2
- 1. Conidia without *Selenosporella*-like synanamorph *E. liquidambaris*
- 2. Conidia with central cells brown and other cells paler *E. acutispora*
- 2. Conidia brown to dark brown with an apical cell paler 3
- 3. Conidia 3-septate, 18–25 × 8–10 µm *E. acuta*
- 3. Conidia 0–3-septate, 14.5–22 × 4.5–6.5 µm *E. selenosporellaria*

Endophragmiella filiformis Chuaseehar., Somrith. & Boonyuen, sp. nov. FIG. 2
IF 558650

Differs from *Endophragmiella rostrata* in having a *Selenosporella*-like synanamorph.
TYPE: Thailand, Nan Province, Bo Kluea District, a stream near Huai Mafaen Nature Trail, 19.1481°N 101.1567°E, on a submerged twig of an unidentified plant, 14 January 2016, S. Sommai (Holotype, BBH 49149).

ETYMOLOGY: Latin, *filiformis*, referring to the filiform shape of the conidial appendage.



FIG. 2. *Endophragmiella filiformis* (holotype, BBH 49149): A, B. Colonies on woody substrate; C. Conidiophore and conidiogenous cell showing successive percurrent extensions; D–F, H. Conidia; G, I. Conidia with lageniform conidiogenous cells on the lateral side (arrows) bearing *Selenosporella*-like synanamorph. Scale bars: A–B = 50 μ m; C–I = 10 μ m.

ASEXUAL MORPH. COLONIES on the natural substratum effuse, hairy, brown. MYCELIUM partially superficial, mostly immersed, smooth-walled, composed of branched, septate, pale brown to brown hyphae, 2–2.5 μ m diam. CONIDIOPHORES macronematous, mononematous, solitary, cylindrical, erect, straight or slightly flexuous, smooth- and thick-walled, simple or occasionally branched, septate, brown, paler near the tip, up to 157.5 μ m high, 2.5–5 μ m wide at the base, with up to 9 or more percurrent extensions at the upper part. CONIDIOGENOUS CELLS monoblastic, integrated, terminal, percurrent, cylindrical, tapering to a truncate apex, pale brown. CONIDIAL SECESSION

rhexolytic. CONIDIA acrogenous, solitary, obclavate, rostrate, smooth-walled, (3–)4(–5)-septate, central cells pale brown, basal cell usually subhyaline but sometimes pale brown, apical cells subhyaline, body of conidium (25–)27.5–30(–32.5) μm long, 5 μm wide at the broadest part (avg. = $28.3 \times 5 \mu\text{m}$, $n = 25$); apex acute with a filiform, aseptate, hyaline appendage up to 10–167.5 μm long; base truncate with a distinct, subhyaline, $0.6\text{--}1.2 \times 2.4\text{--}3.3 \mu\text{m}$ basal frill.

SYNANAMORPH: *Selenosporella*-like. CONIDIA filiform, acerose, aseptate, hyaline, $5\text{--}6 \times 0.3\text{--}0.5 \mu\text{m}$, developed from ampulliform or lageniform conidiogenous cells on the lateral side of the conidia.

SEXUAL MORPH unknown.

COMMENTS—The taxonomic group of *Endophragmiella* species producing rostrate conidia with a long filiform apical appendage includes *E. filiformis*, together with *E. sporoprolifera* (described below; on p. 991), *E. fusiformis* W.P. Wu, *E. nanlingensis* S.C. Ren & X.G. Zhang, *E. pulchra* (B. Sutton & Hodges) P.M. Kirk, *E. rostrata* P.M. Kirk, *E. unisetulata* (Matsush.) S. Hughes, and *E. variabilis* R.F. Castañeda. The main characteristics of *Endophragmiella* species with apical filiform appendages are summarized in TABLE 1.

Apart from possessing the apical filiform appendage, both *E. sporoprolifera* and *E. variabilis* are similar to *E. filiformis* in their conidial shape and pigmentation. However, those two species differ from *E. filiformis* by having Y-shaped conidia. Moreover, conidia of *E. sporoprolifera* are larger and lacking synanamorph ($45\text{--}67.5 \times 10\text{--}12.5 \mu\text{m}$) whereas conidia of *E. variabilis* are smaller ($15\text{--}23 \times 4\text{--}5 \mu\text{m}$; Castañeda-Ruíz 1988). *Endophragmiella filiformis* differs from *E. fusiformis*, *E. nanlingensis*, *E. pulchra* and *E. unisetulata* in having shorter and narrower conidia (Matsushima 1975, Hughes 1979, Kirk 1982b, Wu & Zhuang 2005, Ren & al. 2011). *Endophragmiella rostrata* was originally described by Kirk (1985). Ren & al. (2011) reported a collection of *E. rostrata* from dead branches in China, with shorter, more septate conidia than the type material. The holotype of *E. rostrata* differs from *E. filiformis* in processing its conidia with fewer septa (2–3), different pigmentation (pale brown to brown), smaller size (body of conidium $13\text{--}24 \times 4\text{--}5 \mu\text{m}$, rostrum 25–60 μm) and lacking synanamorph (Kirk 1985). In contrast, the conidial size and the number of septa of *E. filiformis* somewhat overlap with the additional collection of *E. rostrata* from Ren & al. (2011). However, *E. filiformis* can be distinguished from that collection by its conidia showing two different pigmentation patterns and having a longer appendage with the production of synanamorph.

TABLE 1. Comparison of *Endophragmiella* spp. producing conidia with an apical filiform appendage. New species in bold font.

TAXON	CONIDIAL MORPHOLOGY					REFERENCE	
	SHAPE	SEPTATION	PIGMENTATION	BODY (μm)	APPENDAGE/ ROSTRUM (μm)		SYNANAMORPH CONIDIA (μm)
<i>E. filiformis</i>	Obclavate	3–5	Central cells pale brown, basal cell subhyaline or pale brown, apical cells subhyaline	25–32.5 × 5	10–167.5	Sometimes present; filiform or acerose, 5–6 × 0.25–0.5	This study
<i>E. fusiformis</i>	Fusiform	6–7	Medium to dark brown with pale to medium brown end cells	62–80 × 7.5–9	Sometimes present (<85 × 2–2.5)	Absent	Wu & Zhuang 2005
<i>E. nanlingensis</i>	Broadly fusiform	3–5	Central cells brown, basal cells and apical cells pale brown	55–80 × 10–13	23–33	Absent	Ren & al. 2011
<i>E. pulchra</i>	Ellipsoid	3	Median cells brown, basal cell and apical cell pale brown	35–40 (including rostrum) × 5.5–6.5	8–11	Absent	Ren & al. 2011
<i>E. rostrata</i>	Obclavate	2–3	Pale brown to brown	13–24 × 4–5	25–60	Absent	Kirk 1985
<i>E. sporoprolifera</i>	Obclavate	3–4	Pale brown to brown	20–30 × 4–5	25–40	Absent	Ren & al. 2011
	Obclavate, rarely Y-shaped	5–6	Brown; basal cells and apical cells paler	45–67.5 × 10–12.5	55–200	Absent	This study
<i>E. unisetulata</i>	Fusiform	2	Central cell brown, apical and basal cells pale brown	30–36 × 9–10	24–42 × 1	Absent	Matsushima 1975, Hughes 1979
<i>E. variabilis</i>	Obclavate or Y-shaped	2–4	Central cells brown, upper cells and lower cells paler towards the ends	15–23 × 4–5, (branches 9–10 × 3)	28–125	Sometimes present; fusiform, 3–10 × 0.5	Castañeda-Ruiz 1988

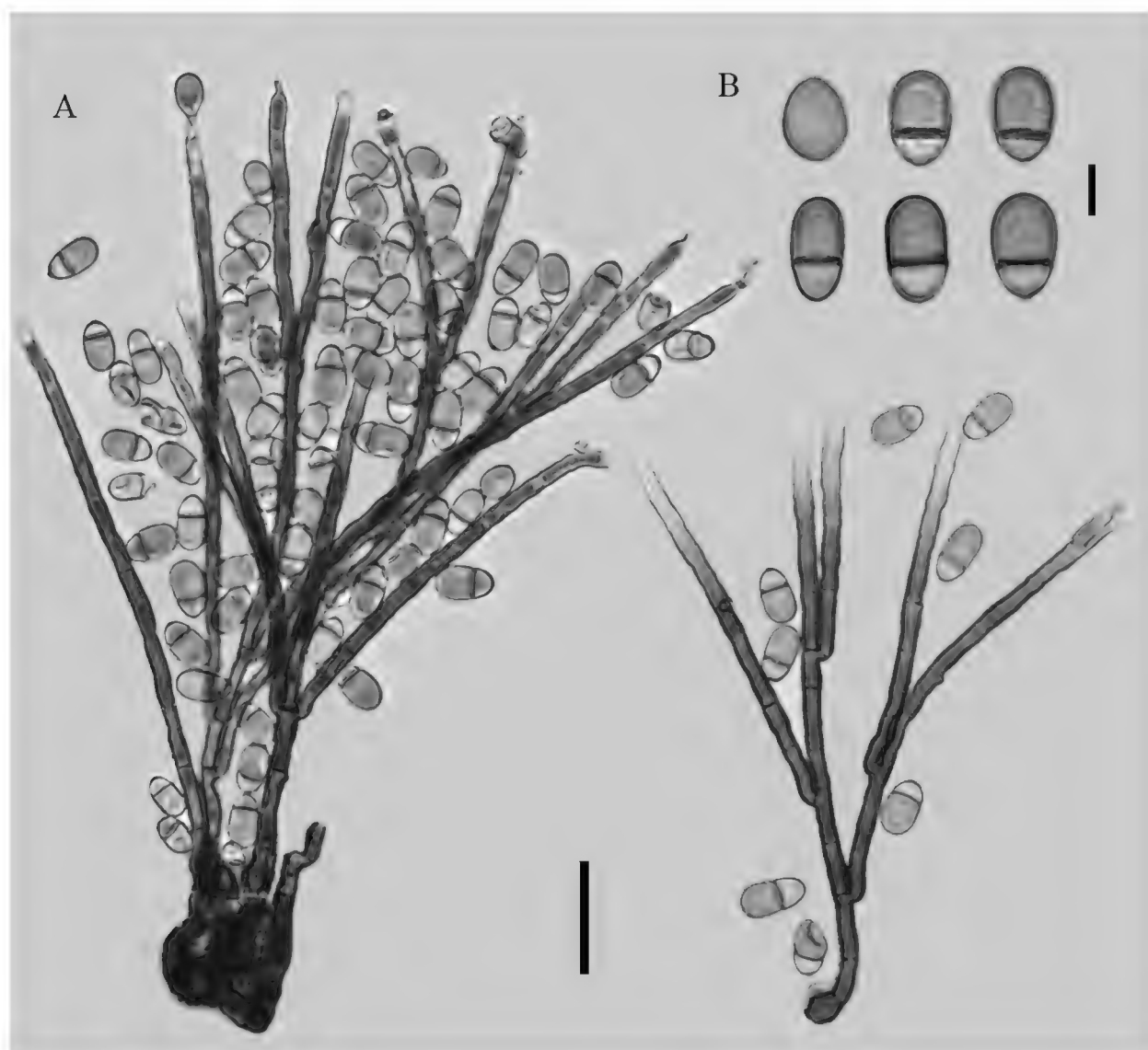


FIG. 3. *Endophragmiella multiramosa* (BBH 49148): A. Conidiophores with compactly branched stromatic base, conidiogenous cells and conidia showing inconspicuous successive percurrent extensions; B. Conidia. Scale bars: A = 20 μm ; B = 5 μm .

ASEXUAL MORPH. COLONIES on the natural substratum effuse, tufted, brown to dark brown. **MYCELIUM** partially superficial, mostly immersed, smooth-walled, composed of branched, septate, pale brown to brown hyphae. **CONIDIOPHORES** macronematous, mononematous, conspicuous caespitose, cylindrical, erect, straight or slightly flexuous, smooth- and thick-walled, compactly branched, septate, brown, paler near the tip, up to 212.5 μm high, 2.5–6 μm wide at the base, with up to 5 or more percurrent extensions at the upper part. **CONIDIOGENOUS CELLS** monoblastic, integrated, terminal, percurrent, cylindrical, tapering to a truncate apex, pale brown. **CONIDIAL SECESSION** rhexolytic. **CONIDIA** acrogenous, solitary, obovoid, ellipsoidal, smooth-walled, usually 1-septate, basal cell pale brown, apical cell brown, 11.3–13.8 μm long, 6.3–7.5 μm wide

at the broadest part, rarely 0-septate, brown, 7.5–8 µm long, 5.8–6.3 µm wide at the broadest part, apex rounded; base truncate with a distinct, subhyaline, 0.8–1.2 × 1.2–1.8 µm basal frill.

SEXUAL MORPH unknown.

SPECIMEN EXAMINED: **THAILAND, BUENG KAN PROVINCE, Si Wilai District**, Nature Trail, 18.1344°N 103.8814°E, on a decaying twig of an unidentified plant, 24 September 2013, C. Chuaseeharonnachai (BBH 49148).

COMMENTS—*Endophragmiella multiramosa* was originally described by Chen & al. (2008) from a rotten twig collected in Taiwan. The new collection (BBH 49148) shares strikingly similar characteristics compared with the type species, i.e., conidial morphology, compactly branched conidiophores and a terrestrial association on dead plant material, except for the variation of the conidiophores. The conidiophores of our collection are markedly longer than those of the typical material (57.6–88 × 2.8–5.6 µm; Chen & al. 2008), although this difference is considered to reflect natural intraspecific variation. This species has not previously been recorded in Thailand; therefore, we identified this new collection as *E. multiramosa* and introduced it here as the first record from Thailand. We also consider *E. multiramosa* as a rare species, as the collection reported herein represents the only one after the original description.

Endophragmiella obpyriformis Chuaseehar., Somrith. & Boonyuen, **sp. nov.**

IF 558652

FIG. 4

Differs from *E. pentaphylacis*, *E. bisbyi*, and *E. suttonii* by its conidia having brown to dark brown at two upper cells.

TYPE: Thailand, Mukdahan Province, Mueang Mukdahan District, a small Stream in Wat Pa Tham Ta Da, 16.6125°N 104.6611°E, on a submerged decaying twig of an unidentified plant, 30 May 2019, N. Boonyuen (**Holotype**, BBH 49153).

ETYMOLOGY: Latin, *obpyriformis*, referring to the obpyriform shape of the conidia.

ASEXUAL MORPH. COLONIES on the natural substratum effuse, hairy, brown to black. MYCELIUM partially superficial, mostly immersed, smooth-walled, composed of branched, septate, pale brown to brown hyphae, 2.5–5.5 µm diam. CONIDIOPHORES macronematous, mononematous, solitary or in groups of 5–7, subulate to cylindrical, erect, straight or slightly flexuous, smooth- and thick-walled, simple or occasionally branched, septate, brown, paler near the tip, up to 240 µm high, 5–8.8 µm wide at the base, with up to 10 or more percurrent extensions at the upper part, arising from a swollen and radially lobed basal cell. Some conidiophores are composed of a thin and pale brown cup, with an appearance resulting from a remaining wall of a



FIG. 4. *Endophragmiella obpyriformis* (holotype, BBH 49153): A. Conidiophores, conidiogenous cells and conidia; B. Conidium attached to conidiophore and bearing lateral regeneration at its basal cell (arrow); C. Conidiophore and conidiogenous cell showing successive percurrent extensions; D. Conidia. Scale bars = 20 μ m.

seceded conidium. CONIDIOGENOUS CELLS monoblastic, integrated, terminal, percurrent, cylindrical, tapering to a truncate apex, pale brown. CONIDIAL SECESSION rhexolytic. CONIDIA acrogenous, solitary, obovoid or obpyriform, smooth-walled, (2–)3-septate, two upper cells brown to dark brown, a lower cell medium brown and a basal cell pale brown, 22–26.3(–28.8) μm long, (7.5–)10–12.5 μm wide at the broadest part (avg. = $24.5 \times 10.6 \mu\text{m}$, $n = 50$); apex rounded; base truncate with a distinct, subhyaline, $1.3\text{--}2.5 \times 2\text{--}2.5 \mu\text{m}$ basal frill and infrequently arising from a lateral, percurrently extending conidiophore which is cylindrical, hyaline with one or more percurrent extensions.

SEXUAL MORPH unknown.

COMMENTS—*Endophragmiella obpyriformis* belongs to the group that have obovoid or obpyriform and predominantly 3-septate conidia, including *E. bisbyi* B. Sutton ex P.M. Kirk, *E. boothii* M.B. Ellis ex P.M. Kirk, *E. clausenae* L.G. Ma & X.G. Zhang, *E. pentaphylacis* L.G. Ma & X.G. Zhang, and *E. suttonii* P.M. Kirk. However, *E. obpyriformis* differs from these species in conidial size and/or conidial pigmentation pattern. The conidia of the new species are larger than those of *E. bisbyi* ($12.5\text{--}16 \times 5.4\text{--}7.6 \mu\text{m}$; Sutton 1973, Hughes 1979) but smaller than those of *E. clausenae* ($31.5\text{--}42.5 \times 14\text{--}16.5 \mu\text{m}$; Ma & al. 2011) and *E. pentaphylacis* ($25\text{--}32.5 \times 13\text{--}16.5 \mu\text{m}$; Ma & al. 2011). *Endophragmiella suttonii* has distinctly shorter conidia ($16\text{--}18 \times 9.5\text{--}11 \mu\text{m}$; Kirk 1981b) than *E. obpyriformis*. The conidia of *E. obpyriformis* are brown to dark brown and paler toward the lower cells, while the conidia of *E. clausenae* and *E. boothii* are concolorous (Ellis 1959, Hughes 1979, Wu & Zhuang 2005, Ma & al. 2011). In addition, the conidia of *E. boothii* have thick-walled cells; the apical cell is largest and the other cells taper towards the base. (Ellis 1959, Hughes 1979, Wu & Zhuang 2005).

Moreover, *E. obpyriformis* produces conidiophores from typically radially lobed basal cells, and sometimes several conidiophores can be found with a cupped appearance.

Key to *Endophragmiella obpyriformis* and similar species

- 1. Conidia concolorous 2
- 1. Conidia versicolorous 3
- 2. The apical cell of conidia is largest, obtuse and thick-walled *E. boothii*
- 2. The central cells of conidia are largest; the apical cell is acute and thin
..... *E. clausenae*
- 3. Conidia obovoid to obpyriform 4
- 3. Conidia broadly ellipsoid to obpyriform, $25\text{--}32.5 \times 13\text{--}16.5 \mu\text{m}$.. *E. pentaphylacis*
- 4. Conidia longer than $20 \mu\text{m}$, $22\text{--}28.75 \times 7.5\text{--}12.5 \mu\text{m}$ *E. obpyriformis*

- 4. Conidia shorter than 20 µm 5
- 5. Conidia 12.5–16 × 5.4–7.6 µm *E. bisbyi*
- 5. Conidia 16–18 × 9.5–11 µm *E. suttonii*

Endophragmiella resinae P.M. Kirk. Trans. Br. Mycol. Soc. 76: 78 (1981) FIG. 5

ASEXUAL MORPH. COLONIES on the natural substratum effuse, hairy, brown to black. MYCELIUM partially superficial, mostly immersed, smooth-walled, composed of branched, septate, pale brown hyphae, 2–2.5 µm diam. CONIDIOPHORES macronematous, mononematous, solitary, cylindrical, erect, straight or slightly flexuous, smooth- and thick-walled, simple, septate, brown, paler near the tip, up to 155 µm high, 5.5–7.5 µm wide at the base, with up to 8 or more percurrent extensions at the upper part. CONIDIOGENOUS CELLS monoblastic, integrated, terminal, percurrent, cylindrical, tapering to a truncate apex, pale brown to subhyaline. CONIDIAL SECESSION rhexolytic. CONIDIA acrogenous, solitary, obovoid, obpyriform, smooth-walled, 1-septate, apical cell pale brown, basal cell paler, 15.5–21.3 µm long, 7.5–10 µm wide at the broadest part; apex rounded; base truncate with a distinct, subhyaline, 0.5 × 2.5 µm basal frill.

SEXUAL MORPH unknown.

SPECIMEN EXAMINED: THAILAND, NAKHON RATCHASIMA PROVINCE, Pak Chong District, Nature Trail, 14.4323°N 101.3693°E, on a decaying twig of an unidentified plant, 9 August 2012, S. Sommai (BBH 49147).

COMMENTS—*Endophragmiella resinae* was introduced by Kirk (1981a) and was found on an old wound of *Picea sitchensis* from Great Britain. Ren & al. (2011) reported a new collection (HSAUPH 8346) of *E. resinae* from China (on dead branches of an unidentified plant). Our collection (BBH 49147) shares similar morphological features with the type and the other representative specimens commonly found in terrestrial habitats on dead plant material (Kirk 1981a, Ren & al. 2011).

Endophragmiella resinae is closely related to a group with obpyriform, obovoid or oblong, 1-septate conidia, such as *E. boewei* J.L. Crane ex S. Hughes, *E. bogoriensis* Rifai, *E. bukkensis* Révay, *E. cambrensis* M.B. Ellis, *E. cantabrica* J. Mena & al., *E. dimorphospora* Awao & Udagawa ex P.M. Kirk, *E. multiramosa*, *E. pinicola* M.B. Ellis ex P.M. Kirk, *E. taxi* M.B. Ellis ex P.M. Kirk, *E. tuberculata* S.M. Leão & Gusmão, *E. uniseptata* M.B. Ellis ex P.M. Kirk, and *E. uniseptata* var. *pusilla* Hol.-Jech. (Ellis 1976, Hughes 1979, Holubová-Jechová 1986, Révay 1987, Chen & al. 2008, Rifai 2008, Leão-Ferreira & Gusmão 2010, Hernández-Restrepo & al. 2013).

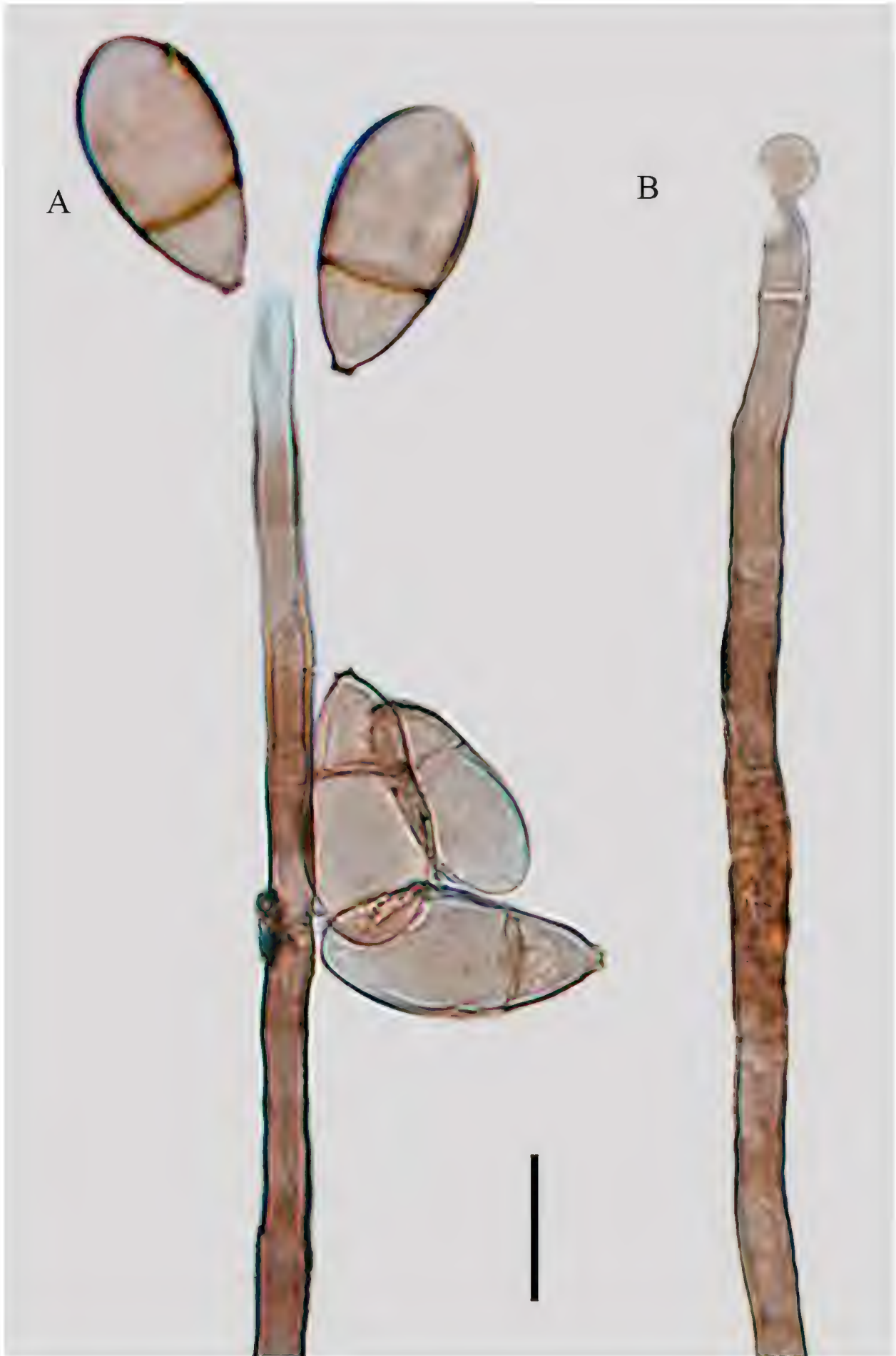


FIG. 5. *Endophragmiella resiniae* (BBH 49147). A. Conidiophore, conidiogenous cell and conidia showing inconspicuous successive percurrent extensions; B. Conidiogenous cells bearing immature conidium. Scale bar = 10 μ m.

It has been mentioned that *E. resinae* clearly differs from other related species by the size of the apical cell, which is typically 1.5–3 times longer than the basal cell (Ren & al. 2011). This species has been reported from Great Britain and China, and this is the first record from Thailand.

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FIG. 6



FIG. 6. *Endophragmiella sporoprolifera* (holotype, BBH 49146). A. Conidiophore and conidiogenous cell with conidium; B. Conidium attached to conidiophore and bearing lateral regeneration at its basal cell (arrow); C. Conidiophore and conidiogenous cell after successive percurrent extensions and attached conidium (arrow); D. Conidiophores and conidiogenous cells; E, F. Conidia; G, H. Y-shaped conidia. Scale bars = 20 μm.

Differs from *E. variabilis* by its larger, 5–6-septate conidia and lacking a *Selenosporella*-like synanamorph.

TYPE: Thailand, Samut Prakan Province, Phra Pradaeng District, Si Nakhon Khuean Khan Park and Botanical Garden, 13.6970°N 100.5645°E, on a decaying twig of an unidentified plant, 1 November 2014, C. Chuaseeharonnachai (**Holotype**, BBH 49146).

ETYMOLOGY: Latin, *sporoprolifera*, referring to the percurrent extensions on the conidiophore of germinated conidia.

ASEXUAL MORPH. COLONIES on the natural substratum effuse, hairy, brown. **MYCELIUM** partially superficial, mostly immersed, smooth-walled, composed of branched, septate, pale brown to brown hyphae, 2.5–7 µm diam. **CONIDIOPHORES** macronematous, mononematous, solitary, cylindrical, erect, straight or flexuous, smooth- and thick-walled, simple or occasionally branched, septate, pale brown to brown, paler near the tip, up to 182.5 µm high, 6.25–8.75 µm wide at the base, with up to 9 or more percurrent extensions at the upper part. **CONIDIOGENOUS CELLS** monoblastic, integrated, terminal, percurrent, cylindrical, tapering to a truncate apex, pale brown to subhyaline. **CONIDIAL SECESSION** rhexolytic. **CONIDIA** acrogenous, solitary, usually obclavate, rarely Y-shaped, rostrate, smooth-walled, 5–6-septate, brown, basal cells and apical cells paler, body of conidium 45–67.5 µm long, 10–12.5 µm wide at the broadest part (avg. = 54.4×12 µm, $n = 25$); apex acute with a filiform, hyaline appendage, up to 55–200 µm long; base truncate with a distinct, subhyaline, $0.8\text{--}1.8 \times 5\text{--}6.25$ µm basal frill, and occasionally with a lateral percurrently extending conidiophore. The lateral conidiophore is cylindrical with up to two or more percurrent extensions.

SEXUAL MORPH unknown.

COMMENTS—*Endophragmiella sporoprolifera* is the eighth species of *Endophragmiella* with a long apical filiform appendage. *Endophragmiella sporoprolifera* is most similar to *E. variabilis* in conidial pigmentation and production of two types of conidia (obclavate and Y-shaped). However, conidia of *E. variabilis* are distinctly smaller (body of conidium $15\text{--}23 \times 4\text{--}5$ µm, branches $9\text{--}10 \times 3$ µm, rostrum 28–125 µm), possess fewer (2–4) septa, and exhibits a synanamorph (Castañeda-Ruíz 1988). *Endophragmiella sporoprolifera* somewhat resembles *E. filiformis* and *E. rostrata* in the obclavate shape of conidia but differs in having more conidial septa and larger conidial sizes (Kirk 1985). *Endophragmiella filiformis* sometimes produces synanamorph, which are not found in *E. sporoprolifera*.

Endophragmiella fusiformis, *E. nanlingensis*, *E. pulchra*, and *E. unisetulata* resemble *E. sporoprolifera* in conidial pigmentation, which is brown in central cells and pale brown in terminal cells. However, *E. sporoprolifera* differs from the others in the shape, size, and septation of conidia (Matsushima 1975, Hughes 1979, Wu & Zhuang 2005, Ren & al. 2011). *Endophragmiella sporoprolifera* is described for the first time from a decaying twig of an unidentified plant in a terrestrial habitat in an area called Bang Kachao, an island in Samut Prakan Province formed by a canal connecting the bend of the Chao Phraya River. It is influenced by high and low tides and salinity levels due to its proximity to the sea. Currently, Bang Kachao is threatened as a habit for saprobic fungi due to rapid urbanization and industrialization.

Morphologically, *Endophragmiella sporoprolifera* is remarkably similar to genus *Teratosperma* Syd. & P. Syd. in having branched conidia. However, *Teratosperma* species have conidia with a lateral branch arising from the basal cell (Ellis 1957, Wu & Zhuang 2005, Kirschner & al. 2019, Xu & al. 2021), while *E. sporoprolifera* has dimorphic conidia with obclavate and Y-shaped which branches arising from suprabasal cells. Furthermore, *E. sporoprolifera* occasionally has the production of secondary conidiophore. The secondary conidiophores formed laterally from the basal cell of the primary conidium, with two (or a few more) annellidic proliferations.

Key to *Endophragmiella filiformis* and *E. sporoprolifera* and similar species

- 1. Y-shaped conidia present 2
- 1. Y-shaped conidia absent 3
- 2. Conidia 5–6-septate, 100–267.5 × 10–12.5 µm (including appendage, 55–200 µm long); without *Selenosporella*-like synanamorph *E. sporoprolifera*
- 2. Conidia, 2–4-septate, 43–148 × 4–5 µm (including rostrum, 28–125 µm long); with *Selenosporella*-like synanamorph *E. variabilis*
- 3. Conidia concolorous, pale brown to brown *E. rostrata*
- 3. Conidia versicolorous 4
- 4. *Selenosporella*-like synanamorph present *E. filiformis*
- 4. *Selenosporella*-like synanamorph absent 5
- 5. Conidia ellipsoid, 35–40 × 5.5–6.5 (including rostrum, 8–11 µm long) . *E. pulchra*
- 5. Conidia fusiform to broadly fusiform 6
- 6. Verrucose-walled conidia present *E. unisetulata*
- 6. Verrucose-walled conidia absent 7
- 7. Conidia 6–7-septate, 147–165 × 7.5–9 (including appendage, up to 85 × 2–2.5 µm) *E. fusiformis*
- 7. Conidia 3–5-septate, 78–113 × 10–13 µm (including rostrum, 23–33 µm long) *E. nanlingensis*

Endophragmiella tunicata Chuaseehar., Somrith. & Boonyuen, **sp. nov.** FIG. 7
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Differs from *E. bicolorata*, *E. bohaniensis*, *E. chinensis*, *E. collapsa*, *E. constricta*, *E. fallacia*, and *E. oblonga* by its having tunica enclosing upper cells of conidia.

TYPE: Thailand, Nakhon Nayok Province, a Waterfall in Pak Phli District, 14.1633°N 101.2683°E, on a submerged twig of an unidentified plant, 3 September 2014, C. Chuaseeharonnachai (**Holotype**, BBH 49145).

ETYMOLOGY: Latin, *tunicata*, referring to the conidia being surrounded by a pale brown tunica.

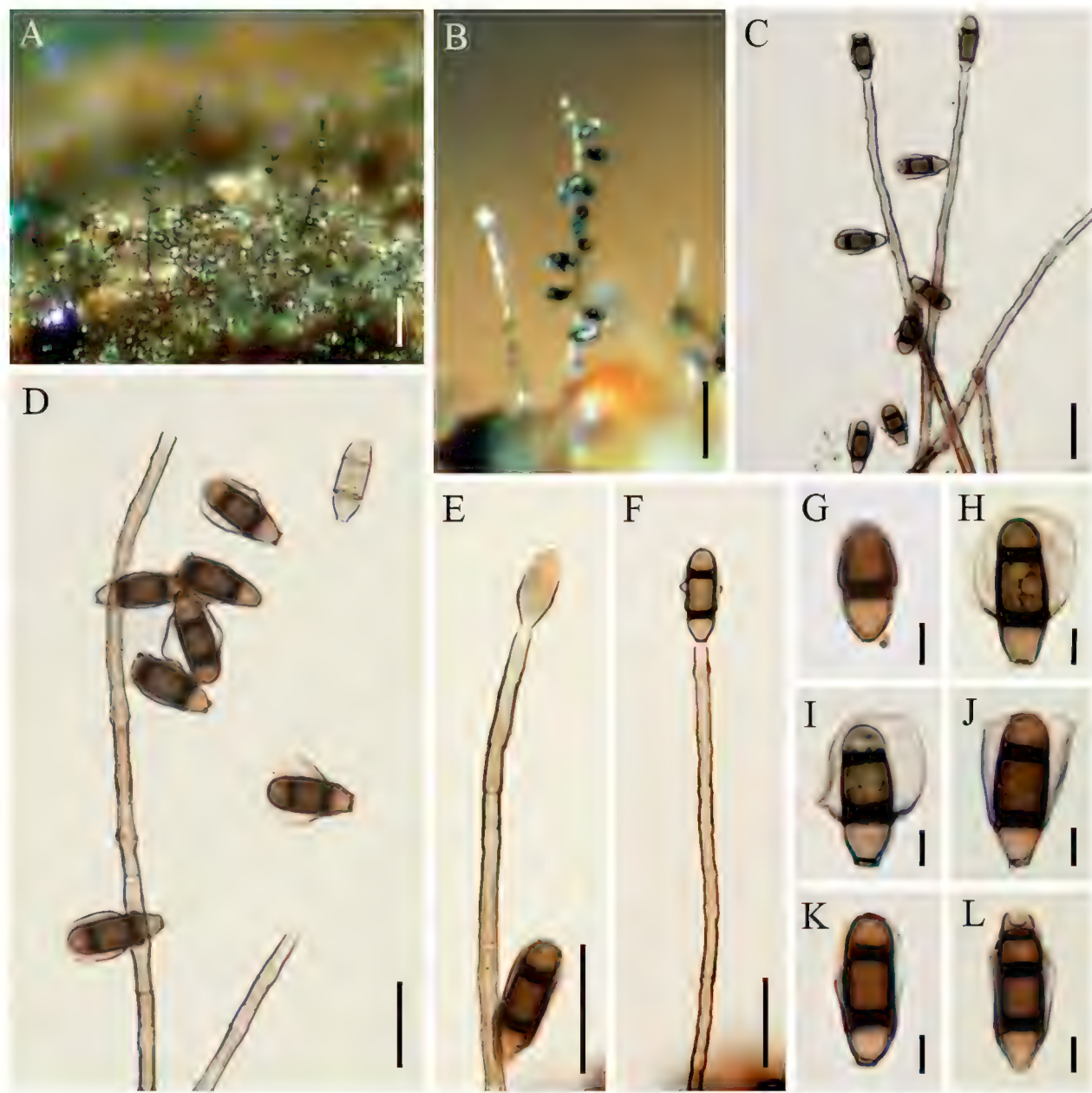


FIG. 7. *Endophragmiella tunicata* (holotype, BBH 49145). A. Colony on woody substrate; B. The seceded conidia remain attached to the lateral surface of conidiophore; C, D. Conidiophores, conidiogenous cells and conidia showing successive percurrent extensions; E. Conidiogenous cells bearing immature conidium; F. Almost mature conidium; G–L Conidia with light brown sheaths. Scale bars: A = 100 µm; B = 50 µm; C–F = 20 µm; G–L = 5 µm.

ASEXUAL MORPH. COLONIES on the natural substratum effuse, hairy, brown to black. MYCELIUM partially superficial, mostly immersed, smooth-walled, composed of branched, septate, pale brown to brown hyphae, 2–5 μm diam. CONIDIOPHORES macronematous, mononematous, solitary, cylindrical, erect, straight or slightly flexuous, smooth- and thick-walled, simple or occasionally branched, septate, brown, paler near the tip, up to 325 μm high, 3.8–7.5 μm wide at the base, with up to 10 or more percurrent extensions at the upper part. CONIDIOGENOUS CELLS monoblastic, integrated, terminal, percurrent, cylindrical, tapering to a truncate apex, pale brown. CONIDIAL SECESSION rhexolytic. CONIDIA acrogenous, solitary, cylindrical or oblong, smooth-walled, (1–)2(–3)-septate, a central cell brown, basal and apical cells paler, 16.3–22.5 μm long, 6.3–8 μm wide at the broadest part (avg. = $20.4 \times 7.4 \mu\text{m}$, $n = 50$), surrounding by a globose to subglobose or obovoid, thin-walled, fragile, light brown, $15\text{--}20 \times 7.5\text{--}12.5 \mu\text{m}$ sheath; apex rounded; base truncate with a distinct, subhyaline, $0.8\text{--}2 \times 3.3\text{--}3.8 \mu\text{m}$ basal frill. A thin sheath arising from the conidial wall at the basal septum or below, usually enclosing 2 upper cells (rarely 3 upper cells) of the conidium or broken at the tip resulting in a cup shape. SEXUAL MORPH unknown.

COMMENTS—*Endophragmiella tunicata* is considered to be in the genus *Endophragmiella* due to its appearance of rhexolytic conidial secession followed by percurrent conidiogenesis. However, *E. tunicata* is unique and differs from other *Endophragmiella* species in having a sheath enclosing the upper cells of the conidia. Therefore, this fungus is described and introduced as a new species, *E. tunicata*.

With the exception of sheath-enclosed conidia, several *Endophragmiella* taxa have cylindrical or oblong conidia with two septa that resemble *E. tunicata*. These species are *E. bicolorata* Hern.-Restr. & al., *E. bohaniensis* N.D. Sharma, *E. chinensis* L. Qiu & al., *E. collapsa* B. Sutton ex P.M. Kirk, *E. constricta* M.T. Dunn, *E. fallacia* P.M. Kirk, and *E. oblonga* Matsush. ex S. Hughes. However, *E. tunicata* differs from *E. bicolorata*, *E. bohaniensis*, *E. fallacia*, and *E. oblonga* in conidial size. The conidia of *E. tunicata* are shorter and narrower than those of *E. bicolorata* ($19\text{--}26.5 \times 7.5\text{--}13 \mu\text{m}$; Hernández-Restrepo & al. 2013), *E. bohaniensis* ($18\text{--}33 \times 8.5\text{--}12.5 \mu\text{m}$; Sharma 1985), *E. fallacia* ($16\text{--}30 \times 9\text{--}13 \mu\text{m}$; Kirk 1981b), and *E. oblonga* ($19\text{--}28 \times 9.5\text{--}11.5 \mu\text{m}$; Matsushima 1975, Hughes 1979).

Amongst species with overlapping ranges of conidial size, *E. tunicata* differs from *E. chinensis*, *E. collapsa*, and *E. constricta* by its conidial cell pigmentation pattern. *Endophragmiella collapsa* forms versicolorous conidia with pale brown

to dark brown upper cells and a lower cell that is paler and sometimes collapses (Sutton 1973, Hughes 1979). Conidia of *E. constricta* are versicolorous with brown upper cells and a paler lower cell, and a constricted septum is present at the median part of the conidium (Dunn 1982). Conidia of *E. chinensis* are concolorous and medium brown (Ma & al. 2021a).

Regarding habitats, the new taxon *E. tunicata* was found on woody substratum in a freshwater habitat. *E. constricta* was recorded as a secondarily opportunistic colonizer on the sclerotia of *Sclerotinia minor* Jagger, whereas other morphologically similar species were mostly saprobic species on terrestrials hosts: *E. bicolorata* on dead wood in Spain; *E. bohaniensis* on dead *Lawsonia inermis* wood in India; *E. chinensis* on dead broadleaf branches in China; *E. collapsa* on dead *Alnus* bark in Canada, *E. fallacia* on rotten *Betula pendula* in UK; and *E. oblonga* on rotten *Phyllostachys edulis* stems in Japan (Sutton 1973, Matsushima 1975, Hughes 1979, Kirk 1981b, Dunn 1982, Sharma 1985, Hernández-Restrepo & al. 2013, Ma & al. 2021a).

Key to *Endophragmiella tunicata* with similar species

- 1. Conidia concolorous 2
- 1. Conidia versicolorous 3
- 2. Conidiophores branched, 83–115 × 2.5–4 µm; conidia oblong or cylindrical, 11–21 × 4.5–7.5 µm *E. chinensis*
- 2. Conidiophores unbranched, 75–300 × 5–6 µm; conidia broadly ellipsoidal, 18–33 × 8.5–12.5 µm *E. bohaniensis*
- 3. Conidia constricted at the middle cell, 15–23 × 6.9–10.4 µm *E. constricta*
- 3. Conidia not constricted at the middle cell 4
- 4. Conidia with middle cells medium brown to brown and terminal cells paler 5
- 4. Conidia with other pigmentation pattern 6
- 5. Conidia with sheath, 16.3–22.5 × 6.3–8 µm *E. tunicata*
- 5. Conidia without sheath, 19–28 × 9.5–11.5 µm *E. oblonga*
- 6. Conidia more variable in shape with ellipsoidal to oblong, ellipsoidal, obclavate or obovoid, 19–26.5 × 7.5–13 µm *E. bicolorata*
- 6. Conidia less variable in shape 7
- 7. Conidia obpyriform to cylindrical with basal cell often collapsed, 14.4–20 × 7.2–9 µm *E. collapsa*
- 7. Conidia broadly ellipsoid to cylindrical, 16–30 × 9–13 µm *E. fallacia*

Discussion

Most *Endophragmiella* species are common in terrestrial environments. In contrast, three taxa were recorded from freshwater, including *E. bitriseptata* Goh, K.D. Hyde and *E. triseptata* C.K.M. Tsui & al. on submerged wood in

South Africa and Hong Kong, respectively, and *E. profusa* R.F. Castañeda & al. on submerged decaying *Bucida palustris* leaves in Cuba (Hyde & al. 1998, Tsui & al. 2001, Castañeda-Ruiz & al. 2010). In this study, three species were collected from terrestrial areas, while four species were reported from freshwater areas.

Of the three species described here, *E. acutispora*, *E. filiformis*, and *E. sporoprolifera*, they show some morphological characters that deviate somewhat from the holotype of the typical *Endophragmiella* species (*E. pallescens*; Sutton 1973) in having more variable conidial septation, conidia with a long apical cell, the production of *Selenospora*-like synanamorph on conidia (*E. acutispora* and *E. filiformis*), evenly development in the dimorphic conidia in being obclavate and Y-shaped (*E. sporoprolifera*). However, these new taxa show the important characteristics of *Endophragmiella* by their presence of solitary, holoblastic, acrogenous, septate, dematiaceous conidia with a small basal frill from monoblastic, integrated, terminal, percurrently extending conidiogenous cells on mononematous, simple or branched, dematiaceous conidiophores and rhexolytic conidial secession that warrant their inclusion in *Endophragmiella*.

Endophragmiella species with a long acrose appendage include *E. filiformis*, *E. fusiformis*, *E. nanlingensis*, *E. pulchra*, *E. rostrata*, *E. sporoprolifera*, *E. unisetulata*, and *E. variabilis* (Matsushima 1975; Hughes 1979; Kirk 1982b, 1985; Castañeda-Ruiz 1988; Wu & Zhuang 2005, Ren & al. 2011) are somewhat similar to some taxa in *Alternaria* sect. *Porri* D.P. Lawr. & al. (see Woudenberg & al. 2014) by possessing obclavate, multiseptate and pigmented conidia with a long filiform beak at the apical end on simple or branched and cylindrical conidiophores. However, conidia of these *Endophragmiella* are phragmosporous, solitary, and euseptate on terminal and percurrently extending conidiogenous cells, while conidia of *Alternaria* sect. *Porri* are dictyosporous or phragmosporous, solitary or arranging in short acropetal chains, and disto- and euseptate on terminal or intercalary and non-extending conidiogenous cells. Conidial secession of *Endophragmiella* is rhexolytic, while that of *Alternaria* sect. *Porri* is schizolytic (Seifert & al. 2011, Lawrence & al. 2013).

Endophragmiella obpyriformis and *E. sporoprolifera* share an interesting feature of their conidia, which show a lateral percurrently extending conidiophore while still attached to the conidiogenous cell. This conidial feature closely resembles those of *E. corticola* P.M. Kirk, *E. fatrensis* Hol.-Jech., *E. hymenochaetica* S. Hughes, and *E. lignicola* S. Hughes (Hughes 1978, 1979; Kirk 1982a; Holubová-Jechová 1986). Kirk (1982a) divided the characteristic of conidiophore proliferation into two types: first, the typical proliferation type, as seen in many *Endophragmiella* species in which the conidiophore proliferates

through the apex of the terminal conidiogenous cell; second, the lateral proliferation type, as seen in *E. corticola*, in which conidiophore proliferation arises from the lateral wall of the terminal conidiogenous cell. Unlike the two types mentioned by Kirk (1982a), the proliferation of *E. obpyriformis* and *E. sporoprolifera* occurs at the basal cell of the conidium that is still adhered to the conidiophore.

Endophragmiella appears morphologically similar with other hyphomycete genera in shape, pigmentation, conidial ontogeny and rhexolytic conidial secession to several existing genera associated with such as *Acrophragmis* Kiffer & Reisinger, *Blastophragmia* Jian Ma & al., *Chaetendophragmia* Matsush., *Stratiphoromyces* Goh & K.D. Hyde and *Teratosperma*. However, *Acrophragmis* distinguishes by its conidia presenting a ring of small knobs at central cell (Kiffer & Reisinger 1970); *Blastophragmia* distinguishes by its conidia bearing setulae (Ma & al. 2021b); *Chaetendophragmia* distinguishes by its conidia containing lateral appendages at the middle cells (Matsushima 1971); *Stratiphoromyces* distinguishes by its conidiogenous cells with crowded repeating percurrent extensions that lead to multilayers of wall remnants at the apex of the conidiophores producing conidia with setulae aggregating in a mass (Goh & Hyde 1998) and *Teratosperma* distinguishes by its conidia with lateral branches arising from the basal cell (Sydow & Sydow 1909). None of these similar genera have DNA sequence data for elucidating their phylogenetic relationships and therefore the differences in generic circumscription between these genera cannot be examined by molecular data at the moment.

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